42

Department of Agriculture
and Fisheries for Scotland

Genetic Protein Variation in Farmed Atlantic Salmon in Scotland: Comparison of Farmed Strains with their Wild Source Populations

Scottish Fisheries Research
Report Number 42 1989
ISSN 0308 8022

LIBRARY
SEP 2 8 1989
National Marine Fisheries Service
2570 Dole Street
Honolulu, Hawaii

A F Youngson
S A M Martin
W C Jordan and
E Verspoor

PREVIOUS SCOTTISH FISHERIES RESEARCH REPORTS

1975 No. 1 The Aberdeen Single-Anchor Mooring.
By R Payne, 7pp.

No. 2 The Aberdeen Shallow Water Current Meter Mooring.
By R. Payne, 13pp.

1976 No. 3 The Bridger Version of the Gulf III High Speed Plankton Sampler.
By J.A. Adams, 42pp.

No. 4 Some Parasites of Plaice *Pleuronectes platessa* L. in Three Different Farm Environments.
By K. MacKenzie, A.H. McVicar and I.F. Waddell, 14pp.

No. 5 Simulation of Vertical Structure in a Planktonic Ecosystem.
By J.H. Steele and E.W. Henderson, 29pp.

No. 6 The Intertidal Fauna of Sandy Beaches — a Survey of the Scottish Coast.
By A. Eleftheriou and A.D. McIntyre, 61pp.

No. 7 A Net Drag Formula for Pelagic Nets.
By A.J. Reid, 12pp.

1977 No. 8 The Aberdeen Sedimentation Trap and its Moorings.
By R. Payne and J.M. Davies, 11pp.

No. 9 A Preliminary Model of Shear Diffusion and Plankton Populations.
By G.T. Evans, J.H. Steele and G.E.B. Kullenberg, 20pp.

No. 10 Diving Observations on the Efficiency of Dredges used in the Scottish Fishery for the Scallop, *Pecten maximus* (L.).
By C.J. Chapman, James Mason and J.A.M. Kinnear, 16pp.

1978 No. 11 Comparative Fishing for Flatfish using a Beam Trawl fitted with Electric Ticklers.
By P.A.M. Stewart, 10pp.

No. 12 The Value of Direct Observation Techniques by Divers in Fishing Gear Research.
By J. Main and G.I. Sangster, 15pp.

1979 No. 13 The Occurrence of Plerocercoids of *Diphyllobothrium* spp. in Wild and Cultured Salmonids from the Loch Awe Area.
By R. Wootten and John W. Smith, 8pp.

No. 14 A Study of Bottom Trawling Gear on both Sand and Hard Ground.
By J. Main and G.I. Sangster, 15pp.

No. 15 The Coastal Movements of Returning Atlantic Salmon, *Salmo salar* L.
By A.D. Hawkins, G.G. Urquhart and W.M. Shearer, 15pp.

No. 16 Hydrodynamic Characteristics of Trawl Warps.
By D.N. MacLennan, 10pp.

No. 17 Some Parasites and Diseases of Blue Whiting, *Micromesistius poutassou* (Risso) to the North and West of Scotland and at the Faroe Islands.
By K. MacKenzie, 14pp.

1980 No. 18 Changes in the Respiration and Blood Circulation of Cod, *Gadus morhua* L., induced by Exposure to Pollutants.
By A.D.F. Johnstone and A.D.Hawkins, 18pp.

1981 No. 19 Survey of Trace Elements in Fish and Shellfish landed at Scottish Ports 1975–76.
By I.M. Davies, 28pp.

No. 20 A Study of the Sand Clouds Produced by Trawl Boards and their Possible Effect on Fish Capture.
By J. Main and G.I. Sangster, 20pp.

Department of Agriculture
and Fisheries for Scotland

Genetic Protein Variation in Farmed Atlantic Salmon in Scotland: Comparison of Farmed Strains with their Wild Source Populations

A F Youngson
S A M Martin
W C Jordan and
E Verspoor

Scottish Fisheries Research
Report Number 42 1989
ISSN 0308 8022

Genetic Protein Variation in Farmed Atlantic Salmon in Scotland. Comparison of Farmed Strains with their Wild Source Populations

by

A F Youngson, S A M Martin, W C Jordan and E Verspoor

Marine Laboratory
Aberdeen

Introduction

Atlantic salmon have been reared in hatcheries over the last century in Scotland, as in other countries, with the objective of augmenting natural smolt production. Commonly, the progeny of wild adults captured locally have been planted out soon after hatching, and without further care. The progeny of non-local fish have sometimes been introduced in the same way, in attempts to alter the biological characteristics of subsequent adult returns to the stocked location.

In general, it is unlikely that past hatchery work has permanently altered the genetic constitution of wild fish in Scotland. Only a small proportion of adults have been stripped in hatcheries each year and this will have imposed limits on any effects of this kind (Calderwood, 1930). Changes may have occurred locally but it is unlikely that this will have happened where stocked alevins have competed with the progeny of natural spawners in streams already filled to capacity. This situation is common in Scotland. In the Girnock Burn, a tributary of Aberdeenshire Dee, for instance, only a fraction of the average annual escape of adults is sufficient to ensure that the stream reaches its carrying capacity for juveniles (Buck and Hay, 1983).

Recently, the commercial culture of Atlantic salmon has become a major industry in Scotland and elsewhere. The numbers of fish in culture are large and increase each year. In 1988, 21 million smolts were produced for sea water culture in Scotland (DAFS, 1988). This number exceeds estimates of the average annual production of wild salmon in Scotland as a whole (R. Gardiner, personal communication).

Cultured fish may live out their entire life-cycle in captivity and their deliberate or inadvertent release to the wild may now be extended from post-hatch juveniles to any of the later, less vulnerable stages of development. These later releases may contribute proportionately more to natural spawning because of increased survival to adulthood.

The development of culture has also made it possible to exercise control on the breeding of successive generations of the same founding group. This power has been used to establish strains of salmon within individual commercial units which are now identified by the name of their river or origin or by the name of the fish farm at which they originated. The genetic constitution of these strains may differ from the constitution of their wild source population. Directionally stable genetic change may result from deliberate or inadvertent selection: any change established by chance in one generation may be sustained in the strain thereafter. Thus, the level of genetic variability of Atlantic salmon in culture is often lower than that present in wild fish (Vuorinen, 1982; Cross and King, 1983; Ståhl, 1983; Verspoor, 1988), probably as a result of random changes caused by the use of small numbers of founding broodstock (Verspoor, 1988).

Reduced genetic variability in cultured fish may lead to immediate reductions in individual performance (Allendorf and Ryman, 1987). It may also lead to reductions in the capacity of groups of fish to respond to novel selective pressures. Furthermore, cultured fish may compete successfully with wild fish following accidental or deliberate release. Their progeny or the progeny of their crossing with wild adults may have a reduced capacity to respond to particular selective pressures acting in the natural environment.

The genetic constitution of Atlantic salmon has been shown to differ between localities (Stahl, 1981, 1983, 1987; Verspoor, 1988). It has been considered that the accuracy of homing in the species has supported the development of local genetic stocks which are uniquely adapted to their own environments (Saunders, 1981; Thorpe and Mitchell, 1981). Conservative advice for those engaged in the enhancement of depleted salmon populations places importance on the effective use of the progeny of any remaining indigenous fish. This approach is most likely to conserve the genetic character of any stock and its intrinsic variability, ensuring its continued stability over long periods of time.

Even if indigenous fish are particularly suited to their own environment they may be disadvantaged by the introduction of groups of non-local salmon or genetically modified strains. This might arise, for instance, where density dependent mortality acts equally on juveniles of mixed origin and where the initial abundance of introduced fish is much greater than that of indigenous ones. Subsequently, different selective pressures to which the indigenous genetic stock was particularly adapted may bear heavily on the introduced fish now dominating the population, to the final detriment of the fishery.

Levels of genetic variation in Atlantic salmon in culture in Scotland have not previously been examined. We have therefore assessed levels of variation in 12 Scottish farmed strains of salmon. We have examined six genetic protein loci which are commonly polymorphic in wild salmon in Scotland, using variation at each locus as an independent indicator of overall levels of genetic variability. We have compared levels of variation in wild and cultured fish, emphasising the relationship between the genetic characteristics of strains and the characteristics of the wild populations on which they are stated to have been founded.

Materials and Methods

The genetic constitution of 12 named strains, drawn from throughout the Scottish salmon farming industry, was examined. The strains were sampled at their Scottish source farm. A group of 50 zero-age juveniles was sampled from each strain in July or August 1988. The sample was randomly taken from all the hatchery groups representing the 1988 year class, following the advice of each hatchery manager. Samples were immediately placed on dry-ice and stored subsequently at $-50°C$.

The farmed strains are not named for reasons of commercial confidentiality but have been assigned identifying numbers. Of the 12 strains examined, eight had been derived from salmon captured in Scottish rivers and four from Norwegian salmon. Of the eight Scottish based strains, four had been derived from the River Shin. All the strains had probably been propagated in culture for four generations or more, with one exception (strain 5) which was second generation when sampled.

The enzymes amino-aspartate transferase (AAT), sorbitol deydrogenase (SDH), iso-citrate dehydrogenase (IDH), malic enzyme (ME) and malate dehydrogenase (MDH) were examined by specific staining (Harris and Hopkinson, 1976) of starch gel electropherograms of tissue extracts run according to the methods of Verspoor (1988). For each individual the enzymes AAT, SDH and IDH were examined in extracts of liver. The enzymes MDH and ME were examined in extracts of white muscle.

In individuals, production of each enzyme is dependent on the presence of one or more genetic loci. Each locus comprises two independently inherited alleles. Both alleles function independently, encoding for specific enzyme structures. In the case of polymorphic loci these alleles may encode for enzymes which are structurally dissimilar and these may be detected by their differential mobility under electrophoresis.

In the present study variant alleles were detected at the Aat-4, Sdh-1, Sdh-2, Idh-4, Me-2 and Mdh-3/4 loci, adopting the nomenclature agreed upon at the Workshop on Genetic Protein Variation in Atlantic Salmon (Verspoor, Cross, Ståhl and Ferguson, in preparation). At the Aat-4 locus, three alleles were scored and the frequencies of the two less common of these are presented. All the alleles found to be present have been described previously (Cross and Ward, 1980). Allele frequency data have been expressed for each strain as the frequency of the occurrence of the allele which is less commonly present in salmon generally (Cross and Ward, 1980). Overall, comparisons of genetic variability could be made on the basis of frequency differences if seven alleles occuring at six loci encoding for five enzymes. These comparisons are probably independent since the available data suggest that the six loci are not associated by linkage (Johnson, 1984; Ståhl, 1987).

Tests of statistical significance have been made by parametric or non-parametric tests in common usage. Additionally, the G-Test and Bartlett's Test are described by Sokal and Rohlf (1981). F_{IS} values were determined according to Wright (1931), using Levine's (1949) correction for sample size.

Results

Strains Differ Genetically from Each Other

Allele frequency variation shows that there are genetic differences between strains (Table I). Significant heterogeneity among all 12 strains was found at each of the six loci examined (G-Test; $P<0.01$). Significant heterogeneity was also present at two (Me-2 and Sdh-2) of the three testable loci for the four strains based on the single River Shin (G-Test; $P<0.01$); the third testable locus (Aat-4) showed heterogeneity at a lower level of significance (G-Test; $P<0.025$).

In none of the strains could the proportions of the various genotypes be considered to differ significantly from the Castle-Hardy-Weinberg distribution expected on the basis of random mating (χ^2 Test). However, the signs of F_{IS} values (Wright, 1931) were determined for each variable locus in every strain (Table II). A negative F_{IS} value is indicative of an excess of heterozygotes at any locus and a positive value of a deficiency. The F_{IS} value was negative in 35 of the 46 possible determinations. The corresponding number anticipated on the basis of the sample sizes and allele frequences is 27.8 (Majumder and Chakraborty, 1981). The difference between the actual and expected number is significant (χ^2 Test; $P<0.03$).

Table I
Allele frequencies at six polymorphic loci in 12 Scottish strains of farmed Atlantic salmon

Strain	N*	Me–2 125	Aat–4 50	Aat–4 25	Sdh–1 28	Sdh–2 –72	Mdh–3 75/80	Idh–3 116	H
Scottish Non-Shin									
1.	50	.580	.080	.030	.000	.520	.020	.060	.223
5.	50	.640	.160	.010	.100	.160	.000	.070	.221
8.	50	.490	.030	.000	.010	.630	.000	.000	.174
9.	50	.760	.060	.000	.000	.750	.000	.000	.142
Shin									
2.	50	.790	.060	.000	.000	.150	.000	.020	.123
3.	50	.450	.140	.040	.030	.410	.000	.010	.227
10.	50	.550	.290	.010	.060	.310	.000	.000	.244
12.	50	.540	.140	.040	.000	.420	.010	.050	.234
Shin mean		.583	.158	.022	.022	.347	.003	.020	.207
Norwegian									
4.	49	.582	.112	.082	.041	.316	.010	.071	.247
6.	50	.690	.200	.000	.051	.480	.030	.040	.246
7.	50	.620	.030	.010	.070	.490	.050	.030	.222
11.	50	.630	.000	.000	.030	.650	.010	.167	.213

* N = number of salmon in sample

Table II
Results of G-tests (df=1) for Castle-Hardy-Weinberg equilibrium of allelotype proportions of 12 Scottish strains ofAtlantic salmon

Loci have been tested in strains when the frequency of the common allele is less than 0.96. Values are presented as $F_{IS} = 1$ (observed heterozygotes/expected heterozygotes). The expected number of heterozygotes has been calculated using Levine's (1949) correction for sample size. The probability of observing an excess of heterozygotes at the given allele frequency and sample size (Majumder and Chakraborty, 1981) is given in parentheses. Significance is denoted as follows: * = P<0.05, ** = P<0.01.

Strain	Me–2	Aat–4	Sdh–1	Sdh–2	Mdh–3	Idh–3
1	–0.22 (.50)	–0.11 (.60)	–	–0.03 (.48)	–	–0.05 (.82)
2	–0.13 (.53)	–0.05 (.82)	–	–0.02 (.54)	–	–
3	0 (.48)	–0.21 (.53)	–	–0.02 (.50)	–	–
4	+0.13 (.51)	–0.23* (.52)	–0.03 (.83)	+0.11 (.51)	–	–0.07 (.77)
5	–0.03 (.52)	–0.05 (.53)	+0.34* (.63)	+0.12 (.53)	–	–0.06 (.78)
6	–0.16 (.53)	+0.63** (.53)	–0.04 (.84)	–0.01 (.51)	–	–0.03 (.84)
7	–0.18 (.51)	–0.03 (.84)	+0.24 (.78)	–0.07 (.47)	–0.04 (.84)	–
8	+0.09 (.47)	–	–	+0.28* (.52)	–	–
9	–0.09 (.53)	–0.05 (.82)	–	+0.10 (.53)	–	–
10	–0.08 (.48)	–0.23 (.53)	–0.05 (.82)	–0.34** (.53)	–	–
11	–0.06 (.52)	–	–	<–0.01 (.53)	–	–0.19 (.51)
12	–0.04 (.48)	+0.06 (.53)	–	–0.14 (.50)	–	–0.04 (.85)

Scottish Farmed Strains Differ from Wild Scottish Populations

Allele frequencies in the farmed strains of salmon differed from frequencies at the same loci in samples of wild Scottish salmon. Variation in the frequency of alleles at six loci in fish of farmed strains was compared with variation at the same loci in 14 samples of zero-age wild salmon drawn from different rivers distributed throughout Scotland (W.C. Jordan and A.F. Youngson, unpublished data). Most of these samples were obtained in 1987 from areas of intense natural spawning which had not recently been disturbed by stocking. The mean and the variance of the frequency distributions at each locus in wild fish and strains were compared (Table III).

Table III
Significance of differences in allele frequencies between 12 Scottish strains of Atlantic salmon and 14 wild Scottish populations (W.C. Jordan and A.F. Youngson, unpublished data), based on arcsin-transformed data

Test	Me–2 125	Aat–4 50	Aat–4 25	Sdh–1 28	Sdh–2 –72	Mdh–3 75/80	Idh–3 116
Difference in t= means (t-test) p=	3.260 0.003	1.000 0.327	0.770 0.447	0.920 0.368	1.950 0.062	1.700 0.103	2.040 0.053
Difference in χ^2= variances p= (Bartlett's test)	0.260 0.608	1.900 0.168	0.140 0.707	0.400 0.528	3.340 0.067	1.110 0.292	0.270 0.606

Significant allele frequency differences were present at the Me–2 locus (Student's t-Test; P=0.003). Using the same statistical test, near significant differences were present at both the Sdh–2 and Idh–3 loci. The validity of this finding for Sdh–2 is in doubt because of the significance of the difference in the variances of the same allele frequencies, which confounds the use of a parametric statistical test. However, testing the significance of the same difference using an appropriate non-parametric test indicates a similar level of probability for its significance (Mann-Whitney U-test; P=0.10).

Combining the probabilities for the seven independent tests for significance in the differences in allele frequencies between the wild salmon and farmed salmon groups (Fisher, 1954) indicates that overall the genetic difference between farmed and wild salmon is highly significant ($P<0.01$).

There is no evidence of significant difference in the variances of allele frequencies at any locus. At three loci variance is greater among samples of wild fish and, in the other four, lower.

Farmed Strains Differ from Their Source River Populations

The allele frequencies now present in strains differ from those now present in salmon in the rivers on which the strains were based. It has been possible to identify the river of origin of nine of the 12 strains examined. Allele frequency variation in samples from these rivers is presented in Table IV. One strain (strain 9) has been derived from the salmon of a relatively small stream. The stream may have been genetically compromised since then because of the proximity of the fish farm. Allele frequencies in this strain have been compared with those present in the fish of another local river which has not been managed in the same way.

Table IV

Allele frequencies for wild populations of Scottish and Norwegian Atlantic salmon used as sources for Scottish farmed strains (W.C. Jordan and A.F. Youngson, unpublished data; E. Verspoor, unpublished data; Vuorinen and Berg, 1989

River	N	Me–2 125	Aat–4 50	Aat–4 25	Sdh–1 28	Sdh–2 –72	Mdh–3 75/8	Idh–3 116	H
Scotland									
N Esk	54	.397	.150	.000	.019	.350	.000	.160	.253
Shin	50	.559	.160	.100	.040	.210	.000	.030	.229
Conon	50	.490	.200	.010	.020	.150	.000	.000	.182
Beauly	50	.396	.160	.000	.000	.290	.000	.170	.248
Thurso	50	.360	.235	.000	.050	.200	.000	.143	.247
Grimersta	50	.670	.250	.000	.040	.448	.000	.040	.244
Helmsdale	50	.400	.110	.010	.020	.240	.000	.010	.190
Norway									
Namsen	74	.500	.053	.019	.000	.456	.024	.000	.194

The Shin Me–2 frequency value is the weighted mean of juvenile and adult samples (combined N = 110)

Another strain (strain 5) has been equally based on adults from two different rivers. Allele frequencies in this strain have been compared jointly with both source populations.

Based on the combined probabilities of the significance of the differences in the mean allele frequencies of the seven alternative alleles at the six variable loci, all the nine strains tested differ highly significantly from their source populations (Table V).

Table V

Significance levels for tests of differences in allele frequencies between farmed strains of Atlantic salmon and the wild populations from which they have been derived

G-tests are used to test differences at the Me–2, Aat–4 and Sdh–2 loci; Fisher's Exact Probability Test is used to test differences at the Sdh–1, Mdh–3, 4 and Idh–3 loci. The overall significance is tested by combining the probabilities for individual tests using $\chi^2 = -2 \ln P$ (df = 2 x b), where b = the number of probabilities combined.

Strain	Me–2	Aat–4	Sdh–1	Sdh–2	Mdh–3	Idh–4	Combined
1	0.47	.3030	.519	.015	.230	.018	.0036
2	5×10^{-5}	8×10^{-5}	.060	.271	–	.500	$<1 \times 10^{-6}$
3	.071	.2110	.499	.002	–	.311	.0063
4	.213	.0190	.027	.016	.460	.016	5×10^{-5}
5	.001	.7700	4×10^{-4}	.207	–	.618	2×10^{-4}
8	.064	8×10^{-6}	.106	1×10^{-6}	–	3×10^{-5}	$<1 \times 10^{-6}$
9	.160	.0002	.060	1×10^{-5}	–	.060	$<1 \times 10^{-6}$
10	.879	.0023	.374	.108	–	.123	.0107
12	.752	.1740	.060	.001	.385	.360	.0083

On the basis of the six alternative alleles at the five testable loci (Mdh–3/4 has been excluded from the comparison because it is sometimes variable in only one of the wild or farmed groups being compared), overall significant (P=0.046) directional differences exist in allele frequencies in the farmed and wild groups (Table VI). If the Me–2 locus (the only individual locus at which wild and farmed groups differ significantly) is excluded from the combined test, it no longer indicates that a significant directional difference exists. This confirms that allele frequencies at the Me–2 locus differ significantly in a consistent directional manner within the framework of the original multiple comparison.

Table VI

Results of paired t-tests for significance of differences in mean allele frequencies in farmed strains of Atlantic salmon and in the wild populations from which they have been derived

The allele frequencies of Shin based strains have been combined as the mean value and used with allele frequency data from another five strains derived from different river populations. Mdh–3,4 has been excluded from the analysis because only in three cases is the locus polymorphic in both the strain and its wild source population.

	Me–2	Aat–4		Sdh–1	Sdh–2	Idh–3
	125	50	25	28	–72	116
x	0.110	0.070	0.005	–0.002	0.140	0.045
sD	0.057	0.108	0.060	0.052	0.214	0.082
t	4.740	1.600	0.600	0.110	1.600	1.210
p	0.005	0.176	0.578	0.918	0.171	0.293

The mean frequency of the variant form of Me–2 in strains derived from River Shin salmon was greater than its frequency in wild fish taken from the river. In each of the other five independent comparisons of strains and their source river the frequency of Me–2 (125) was also greater in the strain than in its source. In the three Scottish strains based on indeterminate or multiple Norwegian sources, the Me–2 (125) frequency was greater than that reported for salmon of any of the four mid-Norwegian rivers for which genetic data are available (Table VII).

Table VII

Allele frequency data for wild populations of Atlantic salmon in Norway (from Stahl, 1987 and Vuorinen and Berg, 1989

River	Me–2	Aat–4		Sdh–1	Sdh–2	Mdh–3	Idh–4	
	125	50	25	28	–72	75/80	116	H
Orstaelva	.611	.056	.009	.000	.528	.009	.000	.186
Bondalselva	.540	.060	.040	.000	.630	.000	.000	.191
Sokna	.493	.069	.139	.000	.435	.000	.000	.223
Namsen	.500	.053	.019	.000	.456	.024	.000	.194
Mean	.536	.060	.052	.000	.512	.008	.000	.199

In one of the four strains derived independently from River Shin salmon (Table I), the value of H (the mean number of loci at which individuals are heterozygous) was lower by about 50% than the level present in wild Shin fish (Table IV). In three of the other five strains of known origin, the value of H was lower by >25% of the value in appropriate source river sample.

Overall, there was no significant difference in the variance of the allele frequencies among farmed strains and among their source river populations at any of the loci examined (Table VIII).

Table VIII

Variance of allele frequencies of six wild populations of Atlantic salmon and of the farmed strains derived from them

The results of Bartlett's test for the homogeneity of the variances based on arcsin-transformed data are also presented. The allele frequencies of Shin based strains have been combined as the mean value and used with allele frequency data from another five strains derived from different river populations. Mdh–3,4 has been excluded from the analysis because only in three cases is the locus polymorphic in both the strain and its source wild population.

Group	Me–2 125	Aat–4 50	Aat–4 25	Sdh–1 28	Sdh–2 –72	Idh–3 116
Farm var.	0.008	0.003	0.001	0.003	0.048	0.001
Wild var.	0.013	0.005	0.002	0.001	0.014	0.004
χ^2 (df=1)	0.160	0.050	0.080	0.650	1.460	0.140
p=	0.693	0.823	0.779	0.420	0.228	0.708

Discussion

This study has demonstrated, on the basis of allele frequency variation at six polymorphic loci, that strains of salmon in culture in Scotland differ genetically from each other. This finding is to be expected given the variety of source river populations on which they were originally based and because wild populations of salmon have previously been demonstrated to differ genetically (Ståhl, 1981, 1983, 1987; Verspoor, 1988). More importantly, however, four farmed strains independently founded on the adults of a single river (the Shin) have also been shown to differ genetically.

In addition, Scottish farmed strains of salmon have been shown to differ genetically from groups of wild salmon sampled from representative rivers distributed throughout Scotland. While there are no consistent overall differences in the levels of genetic variability, significant differences do exist in the representation of alternative alleles at particular loci.

These differences may have arisen because some Scottish strains have been derived from Norwegian river populations. Populations of wild Norwegian salmon and farmed strains based on these may differ from populations in Scotland because of regional genetic differences (Ståhl, 1987) which have been retained in strains. It is, however, unlikely that this is a major cause of the differences because every strain of known origin has also been shown to differ specifically from its source population. The differences between strains and their source may have been generated in any of the following ways.

Genetic Differences within Rivers

Genetic differences exist between juvenile salmon inhabiting different parts of the same river (Ståhl, 1987). The samples of wild fish procured from specific localities within each river and the samples of adults on which the strains were originally based may have been representative of different genetic components of the same river population. This is, however, unlikely to have been the only major cause of the differences demonstrated to exist between strains and their sources. Given the disparate nature of the sites at which zero-age juveniles were sampled and the variety of sites and times of year at which adults were collected in each river, any sampling mis-match would be expected to generate random genetic differences between strains and their sources. However, in the case of the Me–2 locus the difference between source and strain is consistently in one direction.

Temporal Changes in the Genetic Structure of Wild Populations

Allele frequencies in river populations may have altered with time in such a way as to differ from the frequencies present previously when the strains were founded. This may have been a partial cause of non-directional differences between strains and their sources.

It must also be considered a possible cause of consistent directional differences in allele frequencies at the Me–2 locus between strains and their source. In wild fish differences in allele frequencies at the Me–2 locus appear to show environmental dependence (Verspoor and Jordan, in preparation), the frequency of the Me–2 (125) allele tending to be greater where environmental temperatures are lower. Relationships exist for salmon sampled from different rivers and for salmon sampled in different parts of the same river.

It is the Me–2 (125) allele which is consistently over represented in farmed strains of salmon. On this basis, it is possible that Me–2 (125) allele frequencies in wild fish have been depressed consistently by general environmental warming over the period since farmed strains were established. However, air temperature records in Scotland (Meteorological Office, Edinburgh) and water temperature records from the Shelligan Burn, a tributary of the Tay (P. Shackley, personal communication) do not demonstrate a general warming over the period since 1975.

Moreover, W.C. Jordan and A.F. Youngson (unpublished data) have examined Me–2 allele frequencies in adult salmon ascending a small tributary of the Aberdeenshire Dee. Significant between-year variability was present when the fish were assigned to brood years on the basis of scale reading. The data span only five brood years but no consistent directional change with time was evident.

Passive Selection in Culture

Inadvertent selection in culture must also be considered a possible explanation for the relatively greater representation of the Me–2 (125) allele in farmed strains: allele frequency may have altered in response to some consistent aspect of husbandry. Direct selection may have occurred at the locus, reflecting the adaptive importance of genotype where the enzymes produced as the result of the presence of alternative alleles are functionally as well as structurally different. However, if selection has taken place it is more likely that it has been due to the association of Me–2 locus with some other character (or complex of characters) which has been subject to overt selective pressure.

The fish examined in this study had hatched only several months before. The general heterozygote excess indicated by the signs of the F_{IS} values suggests that selection is taking place in culture even over this short time. F_{IS} values at the same loci in wild fish of similar age appear not to be biased in this way (W.C. Jordan, unpublished data), suggesting that the selective mortality apparent in cultured fish occurs only in the farm environment.

Selection in favour of heterozygous individuals will tend to drive allele frequencies in surviving groups of fish towards intermediate values: the observed difference in the frequency of Me–2 (125) allele in strains and their sources can not have resulted from selection of this kind. However, recent work has suggested that a relationship exists in wild salmon between genotype at the Me–2 locus and certain performance traits. Age at first maturity in individual adults appears to be partly determined in this way (Jordan, Youngson and Webb, in preparation). Differential growth rate in individual juveniles also appears to correlate with Me–2 genotypes (W.C. Jordan and A.F. Youngson, in preparation). Growth and sexual

maturity are probably not independent traits and additional performance characters which vary in the same way may remain to be discovered. Selection in culture for the Me–2 (125) allele may have resulted from passive or deliberate selection for growth-related traits like these.

Conclusions

This study has demonstrated that, irrespective of how they may have arisen, overall genetic differences exist in each comparison of strain and source and that allele frequencies at individual loci sometimes differ markedly in these comparisons. This suggests that the successful re-introduction of fish of farmed strains, even to the river from which the strain was originally derived, will alter the natural frequency of alleles at these loci. The effects of allele frequency changes at single loci on the fitness of wild populations of salmon are unknown.

Most biologically important phenotypic characters are polygenic, resulting from the interactive effects of alleles at many loci. The entire genome of fishes may comprise 10,000–100,000 loci, although it is technically possible to examine only a small proportion of these. It is reasonable to assume however, that the condition of those which can be examined is typical of the condition of the others.

On this basis, the frequencies of the possible allele combinations at the six polymorphic loci we have examined have been compared in two populations of fish. A single farmed strain (Strain 8) has been compared with its source. Allele frequency is fixed in both strain and source at one locus but the other five loci are variable in one or both groups of fish. Considering these five loci, 243 unique allele combinations are possible. Their likely frequency of occurrence in each group has been determined from the frequency of alternative alleles at each locus (Fig. 1). It can be seen that some individuals are uniquely characteristic of the group to which they belong. Indeed, allelale combinations expected to be represented in 26% of the individuals of the wild population on which Strain 8 is based are absent from the strain itself. If it were possible to consider also the other polymorphic loci of the genome (which are probably numbered in hundreds) this difference would almost certainly be compounded. Again, the consequences of introducing cultured fish to a stream population from which they differ by this genetic measure are unknown.

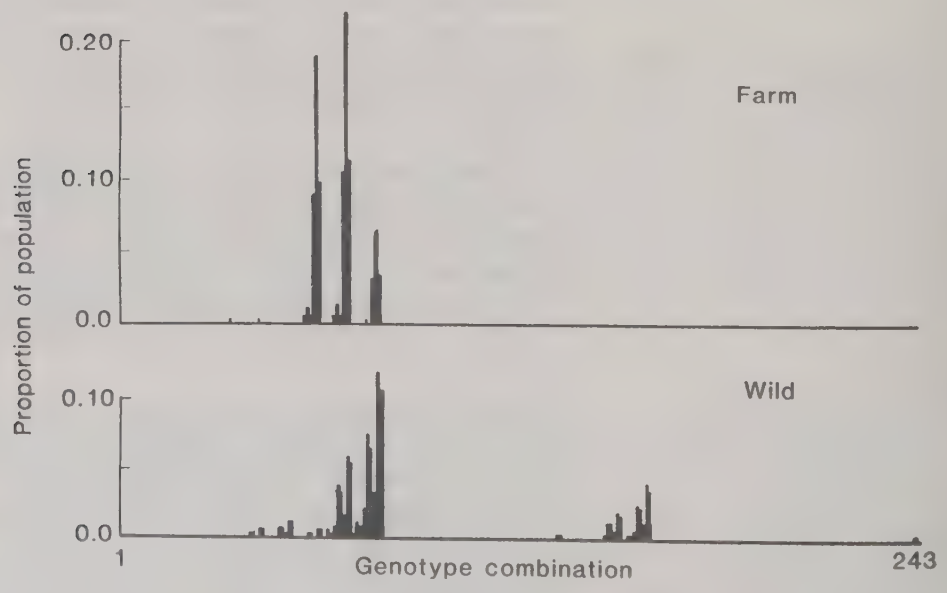

Figure 1. The frequencies of occurrence of unique combinations of alleles at five polymorphic protein loci in a farmed strain of Atlantic salmon (No 8) and in the wild salmon population from which it was originally derived. The frequency of occurrence of allele combinations has been constructed from the frequencies of alternative alleles at single loci. 243 possible allele combinations exist.

Any change in the fitness of wild salmon populations which may result from the introduction of genetically dissimilar fish can be determined only experimentally, in studies which remain to be performed.

We acknowledge the help and guidance of the staff and management of the fish-farms we visited in procuring material for this study. We also acknowledge the support of the District Fishery Boards and their staff in obtaining the samples of wild salmon which made comparison possible.

Summary

Levels of genetic variation in 12 Scottish strains of farmed Atlantic salmon have been assessed by determining allele frequency variation at six polymorphic enzyme loci. The strains differed genetically from each other, and overall they differed from a representative group of wild Scottish populations. In the nine comparisons which could be made, strains also differed genetically from the specific source population from which they had originally been derived. The differences in allele frequencies between strains and their source were consistently in one direction at one locus (Me–2). This can be explained only if selective pressure has been acting consistently on the strains or on the source populations, over the period since the strains were established.

References

Allendorf, F.W. and Ryman, N. 1987. Genetic management of hatchery stocks. *Ryman, N. and Utter, F. eds.* Population genetics and fishery management. Seattle, University of Washington Press, 141–160.

Buck, R.J.G. and Hay, D.W. 1983. The relation between stock size and progeny of Atlantic salmon, *Salmo salar* L., in a Scottish stream. *Journal of Fish Biology,* **24,** 1–12.

Calderwood, W.L. 1930. Salmon and sea trout. London, Edward Arnold and Co., 242pp.

Cross, T.F. and Ward, R.D. 1980. Protein variation and duplicate loci in the Atlantic salmon, *Salmo salar* L. *Genetic Research,* **36,** 147–165.

Cross, T.F. and King, J. 1983. Genetic effects of hatchery rearing in Atlantic salmon. *Aquaculture,* **33,** 33–40.

Fisher, R.A. 1954. Statistical methods for research workers. 12th edition. Edinburgh, Oliver and Boyd, 138pp.

Harris, H. and Hopkinson, D.A. 1976. Handbook of electrophoresis in human genetics. Amsterdam, Elsevier-North Holland.

Johnson, K. 1984. Protein variation in the Salmoninae: genetic interpretations of electrophoretic banding patters, linkage associations among loci, and evolutionary relationships among species. PhD thesis, Pennsylvania State University, Pennsylvania (unpublished), 181pp.

Jordan, W.C., Youngson, A.F. and Webb, J.H. (In preparation). Genetic variation at the Me–2 locus associated with age at maturity in sea-run Atlantic salmon (*Salmo salar*).

Levine, H. 1949. On a matching problem arising in genetics. *Annals of Mathematical Statistics,* **20,** 91–94.

Majumder, P.P. and Chakraborty, R. 1981. Mean and variance of the number of samples showing heterozygote excess or deficiency. *Heredity,* **47,** 259–262.

Saunders, R.L. 1981. Atlantic salmon (*Salmo salar*) stocks and management implications in the Canadian Atlantic provinces and New England, USA. *Canadian Journal of Fisheries and Aquatic Science,* **38,** 1612–1625.

References (Contd.)

Sokal, R.R. and Rohlf, F.J. 1981. Biometry. San Francisco, W.H. Freeman and Company, 859pp.

Ståhl, G. 1981. Genetic differentiation among natural populations of Atlantic salmon (*Salmo salar*) in northern Sweden. *Ryman, N. ed., Fish Gene Pools. Ecological Bulletin (Stockholm)*, **34,** 95–105.

Ståhl, G. 1983. Distribution of genetic variation in natural and hatchery stocks of Atlantic salmon (*Salmo salar*) in northern Europe. *Aquaculture,* **33,** 23–32.

Ståhl, G. 1987. Genetic population structure of Atlantic salmon. *Ryman, N. and Utter, F. eds. Population genetics and fishery management.* Seattle, University of Washington Press, 121–140.

Thorpe, J.E. and Mitchell, K.A. 1981. Stocks of Atlantic salmon (*Salmo salar*) in Britain and Ireland: discreteness and current management. *Canadian Journal of Fisheries and Aquatic Science,* **38,** 1576–1590.

Verspoor, E. 1988. Reduced genetic variability in first generation hatchery populations of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Science,* **45,** 1686–1690.

Verspoor, E., Cross, T.F., Ståhl, G. and Ferguson, A. (In preparation). Nomenclature of genetic protein variation in Atlantic salmon. *Scottish Fisheries Research Report.*

Verspoor, E. and Jordan, W.C. (In preparation). Genetic variation at the Me–2 locus in the Atlantic salmon within and between rivers: evidence for its selective maintenance. *Journal of Fish Biology.*

Vuorinen, J. 1982. Little genetic variation in the Finnish Lake salmon, *Salmo salar sebago* (Girard). *Hereditas,* **97,** 189–192.

Vuorinen, J. and Berg, O.K. 1989. Genetic divergence of anadromous and nonanadromous Atlabtic salmon (*Salmo salar*) in the River Namsen, Norway. *Canadian Journal of Fisheries and Aquatic Sciences,* **46,** 406–409.

Wright, S. 1931. Evolution in Mendelian populations. *Genetics,* **16,** 97–159.

Printed by HMSO Reprographic Unit, Edinburgh
7/89 750 (025195)

1981 No. 21 Orientation and Energetic Efficiency in the Offshore Movements of Returning Atlantic Salmon *Salmo salar* L.
By G.W. Smith, A.D. Hawkins, G.G. Urquhart and W.M. Shearer, 22pp.

No. 22 The Theory of Solid Spheres as Sonar Calibration Targets.
By D.N. MacLennan, 17pp.

No. 23 A Study of the Fish Capture Process in a Bottom Trawl by Direct Observations from a Towed Underwater Vehicle.
By J. Main and G.I. Sangster, 23pp.

1982 No. 24 A Study of Separating Fish from *Nephrops norvegicus* L. in a Bottom Trawl.
By J. Main and G.I. Sangster, 8pp.

No. 25 Target Strength Measurements on Metal Spheres.
By D.N. MacLennan, 11pp.

No. 26 A Study of a Multi-Level Bottom Trawl for Species Separation Using Direct Observation Techniques.
By J. Main and G.I. Sangster, 17pp.

1983 No. 27 Fish Reactions to Trawl Gear — a Study Comparing Light and Heavy Ground Gear.
By J. Main and G.I. Sangster, 17pp.

No. 28 Guidelines for the Use of Biological Material in First Order Pollution Assessment and Trend Monitoring.
By G. Topping, 28pp.

No. 29 TUV II — a Towed Wet Submersible For Use in Fishing Gear Research.
By J. Main and G.I. Sangster, 19pp.

No. 30 The United Kingdom Fishery for the Deep Water Shrimp *Pandalus borealis* in the North Sea.
By F.G. Howard, 16pp.

1984 No. 31 A Selected Review of Hydrodynamic Force Coefficient Data on Stranded Wires Used in Fishing Gear.
By R.S.T. Ferro and E.H. Hou, 20pp.

1985 No. 32 Small Codend Covers.
By P.A.M. Stewart and J.H.B. Robertson, 11pp.

No. 33 Attachments to Codends.
By P.A.M. Stewart and J.H.B. Robertson, 15pp.

No. 34 The Behaviour of the Norway Lobster, *Nephrops norvegicus* (L.), During Trawling.
By J. Main and G.I. Sangster, 23pp.

No. 35 Experiments on the Cultivation of Oysters in Scotland.
By J. Drinkwater and T.R.W. Howell, 20pp.

1986 No. 36 Radio-Tracking Observations on Atlantic Salmon Ascending the Aberdeenshire Dee.
By A.D. Hawkins and G.W. Smith, 24pp.

1987 No. 37 A Model to Assess the Effect of Predation by Sawbill Ducks on the Salmon Stock of the River North Esk.
By W.M. Shearer, R.M. Cook, D.A. Dunkley, J.C. MacLean and R.G.J. Shelton, 12pp.

1988 No. 38 The Intertidal Fauna of Sandy Beaches - A Survey of the East Scottish Coast.
A. Eleftheriou and M.R. Robertson, 52pp.

No. 39 Mesh Selection Within the Cod-end of Trawls. The Effects of Narrowing the Cod-end and Shortening the Extension.
J.H.B. Robertson and R.S.T. Ferro, 11pp.

1989 No. 40 The Movements and Spawning Behaviour of Adult Salmon in the Girnock Burn, A Tributary of the Aberdeenshire Dee, 1986. By John Webb and A.D. Hawkins, 41pp.

No. 41 The Movements of Adult Salmon within the River Spey. By R. Laughton, 19pp.

Available from:–

The Librarian
Department of Agriculture & Fisheries for Scotland
Marine Laboratory
PO Box 101
Victoria Road
Torry, Aberdeen AB9 8DB